

FIGURE 1

(SEQ ID NO:1-nucleotide sequence and SEQ ID NO:2-amino acid sequence)
Escherichia coli B Phytase Sequence

1
 ATG AAA GCG AIC TTA ATC CCA TTT TTA TCT CTT CTG ATT CCG TTA ACC CCG
 Met Lys Ala Ile Leu Ile Pro Phe Leu Ser Leu Leu Ile Pro Leu Thr Pro
 CAA TTT GCA TTC GCT CAG AAT GAG CCG GAG CTG AAG CTG GAA AGT GTG GTG
 Gln Ser Ala Phe Ala Gln Ser Glu Pro Glu Leu Lys Leu Gln Ser Val Val
 ATT GTC AGT CGT CAT GGT GTG CGT GGT CCA AIC AAG GCG ACC GAA CTG ATG
 Ile Val Ser Arg His Gly Val Arg Ala Pro Thr Lys Ala Thr Gln Leu Met
 CAG GAT GTC AIC CCA GAG GCA TGG CCA ACC TGG CTG GTA AAA CTG GCT TGG
 Gln Asp Val Thr Pro Asp Ala Trp Pro Thr Trp Pro Val Lys Leu Gly Trp
 CTG AIA CIG CIG GGT GGT GAG CTA AIC GCG TAT CTC GGA CAT TAC CAA CCG
 Leu Thr Pro Arg Gly Gly Glu Leu Ile Ala Tyr Leu Gly His Tyr Gln Arg
 CAG CTT CTG GTA GCG GAG GCA TTT CIG GCG AAA AAG GCG TCG CCG CAG TCT
 Gln Arg Leu Val Ala Asp Gly Leu Leu Ala Lys Lys Gly Cys Pro Gln Ser
 GGT CAG GTC GCG ATT AAT GCT GAT GTC GAG CAG CGT ACC CTT AAA ACA GCG
 Gly Gln Val Ala Ile Ile Ala Asp Val Asp Glu Arg Thr Arg Lys Thr Gly
 GAA GCG TTC GCG GCG GCG GCG GCG GCA TCT GAG TCT GCA ATA ACC GCA CAT ACC
 Glu Ala Phe Ala Ala Gly Leu Ala Pro Asp Cys Ala Ile Thr Val His Thr
 CAG CCA GAT AAG TCG AAT CCG GAT GAG TTA TTT AAT CCG GCA AAA ACT GCG
 Gln Ala Asp Thr Ser Ser Pro Asp Pro Leu Phe Asn His Leu Lys Thr Gly
 GTT TCG GAA ATG GAT AAG GCG AAC CTC AAT GAG GCG ATC CTC AAG AGG GCA
 Val Cys Gln Leu Asp Asn Ala Asn Val Thr Asp Ala Ile Leu Ser Arg Ala
 GGA GCG GCA ATT GCT GAT TTT ACC GCG CAT CCG GAA AAG GCG TTT CCG GAA
 Gly Gly Ser Ile Ala Asp Phe Thr Gly His Arg Gln Thr Ala Phe Arg Glu
 CIG GAA GCG CTG CTT AAT TTT CCG GAA TCA AAC CTC TCG CTT AAA CCG GAG
 Leu Glu Arg Val Leu Asn Ile Pro Gln Ser Asn Leu Cys Leu Lys Arg Gln
 AAA TCG GAG GAA AAG GGT TCA TTA AIC CAG GCA TTA GCA TCG GAA CTC AAG
 Lys Gln Asp Glu Ser Cys Ser Leu Thr Gln Ala Leu Pro Ser Glu Leu Lys
 GTG AAT GCG GAG AAT CTC TTA TTA AIC GGT CCG GCA AAG CTC GCA TCA ATG
 Val Ser Ala Asp Asn Val Ser Leu Thr Gly Ala Val Ser Leu Ala Ser Met
 CTG ACC GAG ATA TTT CTC CTG GAA GAA GCA CAG GGA AAG CCG GAG CCG GCG
 Leu Thr Glu Ile Phe Leu Leu Gln Gln Ala Gln Gly Met Pro Glu Pro Gly
 TGG GGA AGG ATC ACC GAT TCA CAG CAG TGG AAC ACC TIG GTA AGT TTG CAT
 Trp Gly Arg Ile Thr Asp Ser His Gln Trp Asn Thr Leu Leu Ser Leu His
 AAC GCG CAA TTT TAT TTG CTA CAA CCG ACG GCA GAG ATT GCG CCG ACG TCG
 Asn Ala Gln Phe Tyr Leu Leu Gln Arg Thr Pro Glu Val Ala Arg Ser Arg
 GCG ACC CCG TTA TTG GAT TTG ATC ATG GCA GCG TTG ACG CCG CAT CCA CCG
 Ala Thr Pro Leu Leu Asp Leu Ile Met Ala Ala Leu Thr Pro His Pro Pro

CAA AAA CAG GCG TAT GGT GTG ACA TTA CCC ACT TCA GTA CTG TTT ATT GCC
 Gln Lys Gln Ala Tyr Gly Val Thr Leu Pro Thr Ser Val Leu Phe Ile Ala
 GGA CAC GAT ACT AAT CTG GCA AAT CTC GGC GGC GCA CTG GAG CTC AAC TGG
 Gly His Asp Thr Asn Leu Ala Asn Leu Gly Gly Ala Leu Glu Leu Asn Trp
 ACG CTT CCC GGT CAG CCG GAT AAC ACG CCG CCA GGT GGT GAA CTG GTG TTT
 Thr Leu Pro Gly Gln Pro Asp Asn Thr Pro Pro Gly Gly Glu Leu Val Phe
 GAA CGC TGG CGT CCG CTA ACC GAT AAC AGC CAG TGG ATT CAG GTT TCG CTG
 Glu Arg Trp Arg Arg Leu Ser Asp Asn Ser Gln Trp Ile Gln Val Ser Leu
 GTC TTC CAG ACT TTA CAG CAG ATG CCT GAT AAA ACG CCG CTG TCA TTA AAT
 Val Phe Gln Thr Leu Gln Gln Met Arg Asp Lys Thr Pro Leu Ser Leu Asn
 ACG CCG CCC GGA GAG GTG AAA CTG ACC CTG GCA GGA TGT GAA GAG CGA AAT
 Thr Pro Pro Gly Glu Val Lys Leu Thr Leu Ala Gly Cys Glu Glu Arg Asn
 GCG CAG GGC ATG TGT TCG TTG ACA GGT TTT ACG CAA ATC GTG AAT GAA GCA
 Ala Gln Gly Met Cys Ser Leu Ala Gly Phe Thr Gln Ile Val Asn Glu Ala
 CGC ATA CCG GCG TGC AGT TTG AGA TCT CAT CAC CAT CAC CAT CAC TAA 1323
 Arg Ile Pro Ala Cys Ser Leu Arg Ser His His His His His His End

FIGURE 2
pH/Temperature Profile and Stability

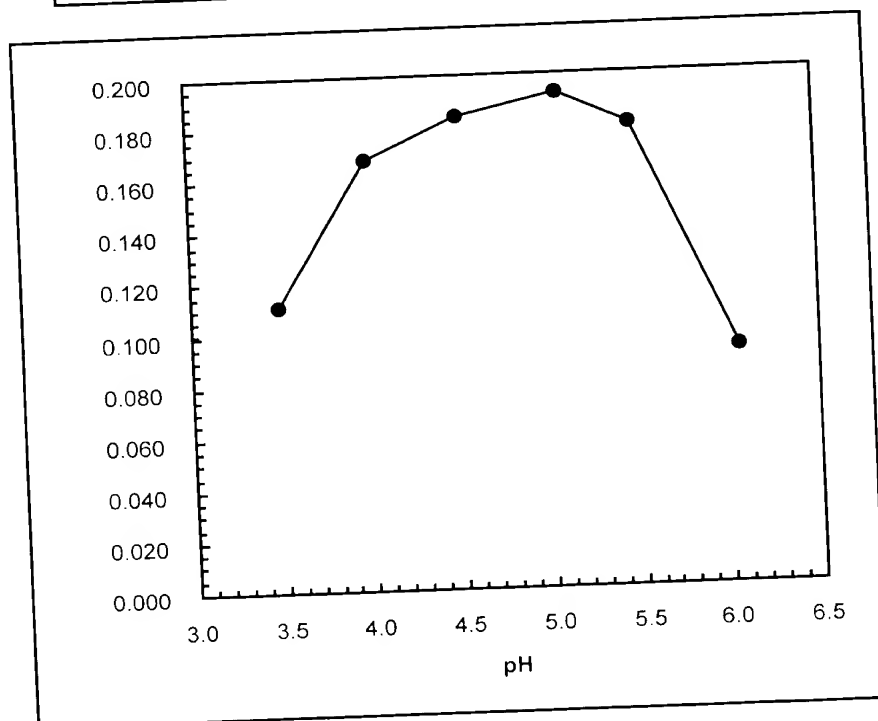
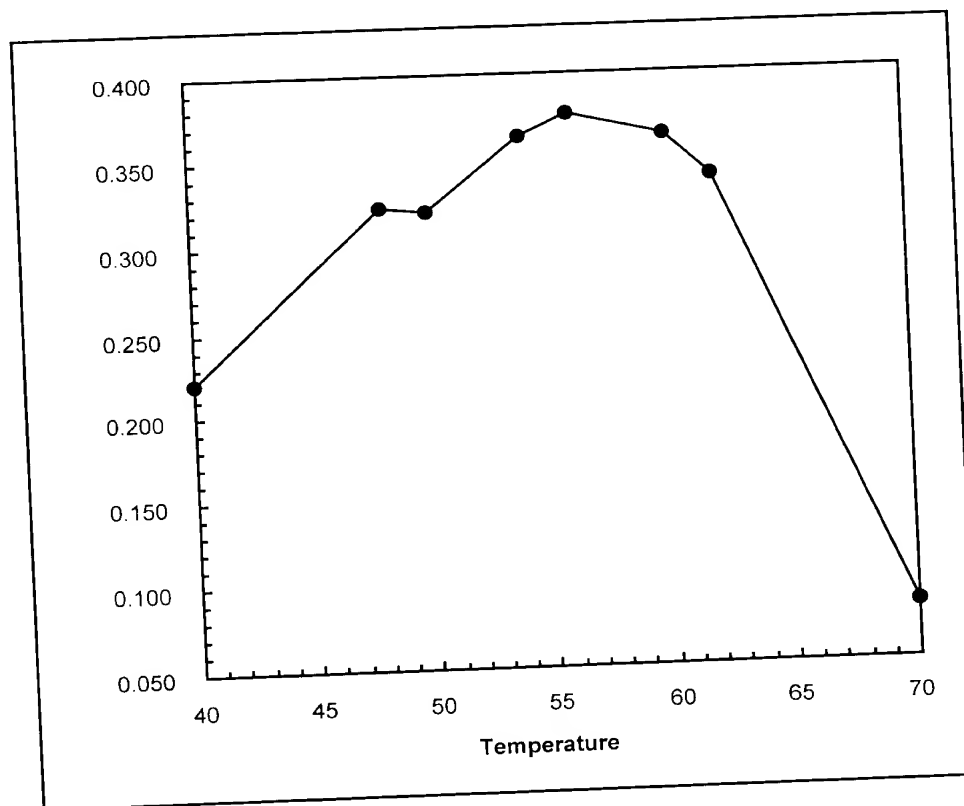


Figure 3:

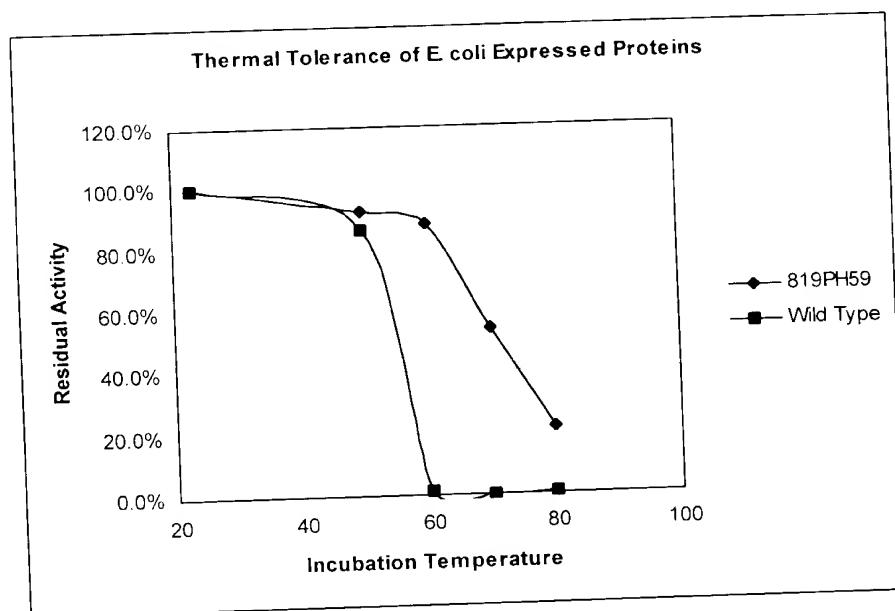


Figure 4

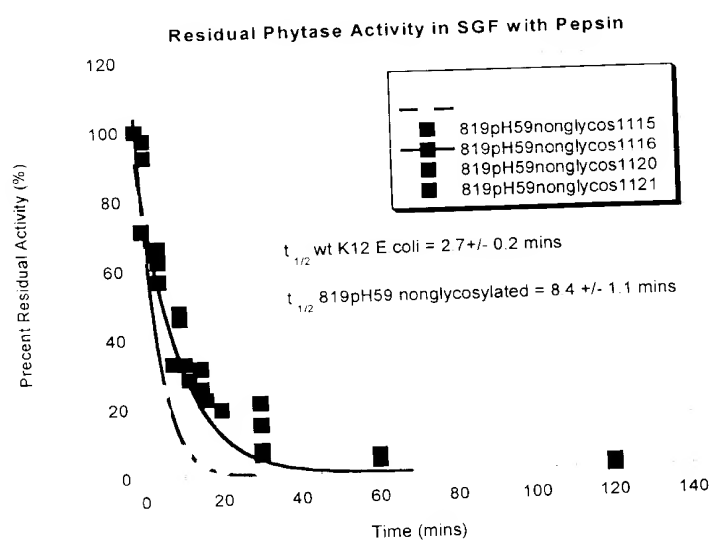


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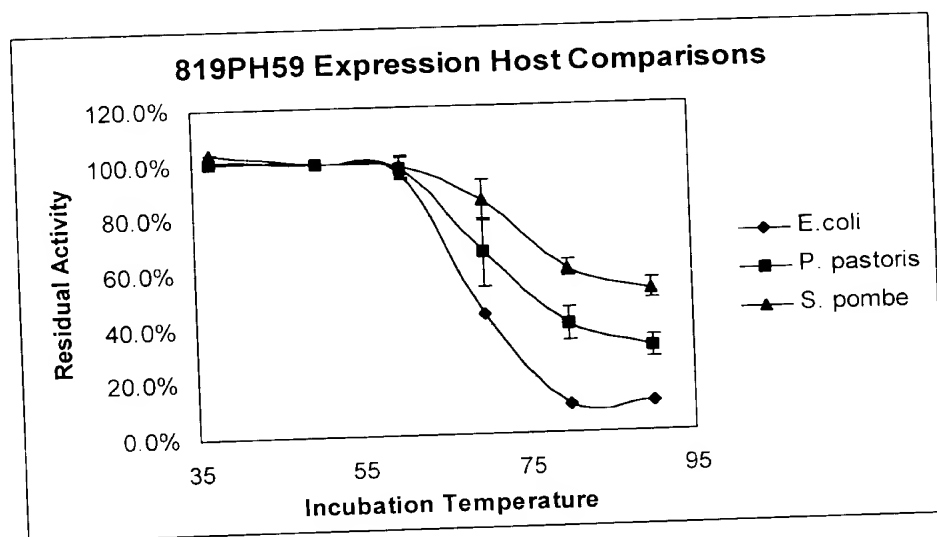


Figure 6.

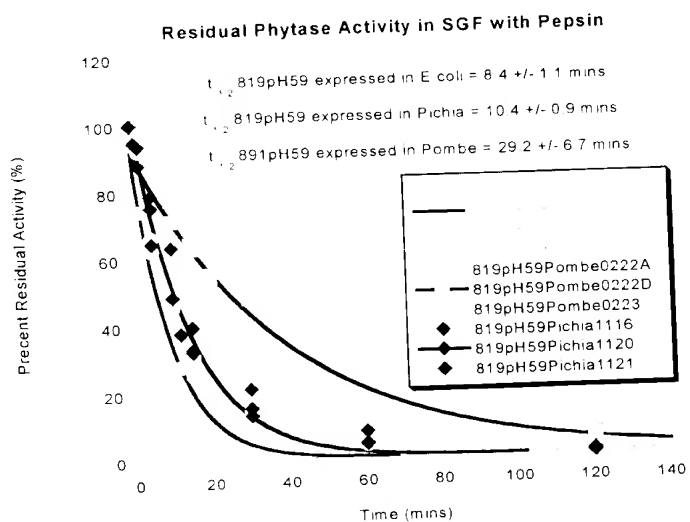


Figure 7a
E. coli appA (GenBank accession no. M58708) (SEQ ID NO:7)

1 taaggagcag aaacaatgtg gtatttactt tggttcgtcg gcattttgtt gatgtgttcg
61 ctctccacce ttgtgttggt atggetggac ccgctctga aaagttaacg aacgtaggcc
121 tgatgcggcg cattagcacc gcatcaggca atcaataatg tcagatatga aaagcgga
181 catatcgatg aaagcgatct taatcccatt ttatctctt ctgattccgt taaccccgca
241 atctgcattc gctcagagtg agccggagct gaagctggaa agtgtggtga ttgtcagtcg
301 tcattggtgtg cgtgtccaa ccaaggccac gcaactgatg caggatgtca cccagacgc
361 atggccaacc tggccggtaa aactgggttg gctgacaccg cngngtggtg agctaategc
421 ctatctcgga cattaccaac gccagcgtct ggtagccgac ggattgctgg cgaanaaggg
481 ctgcccgcag tctggtcagg tccgattat tgcgtatgc gacgagcgta cccgtaaaac
541 aggcgaagcc ttcgccgcg ggctggcacc tgactgtgca ataaccgtac ataccaggc
601 agatacgtcc agtcccgatc cgttatttaa tctctaaaa actggcggtt gccactgga
661 taacgcgaac gtgactgacg cgatcctcag cagggcagga gggtaattg ctgactttac
721 cgggcacggg caaacggcgt ttcgcgaact ggaacgggtg ctttaatttc cgcaatcaaa
781 cttgtgcctt aaacgtgaga aacaggacga aagctgttca ttaacgcagg cattaccatc
841 ggaactcaag gtgagcgcg acaatgtctc attaacgggt gcgtaagcc tgcacatcat
901 gctgacggag atatttctcc tgcaacaagc acagggaatg ccggagccgg ggtggggaag
961 gateaccgat tcacaccagt ggaacacctt gctaagtgtg cataacgcgc aattttatt
1021 gctacaacgc acgccagagg ttgccgcag ccgcgccacc ccgttattag atttgatcaa
1081 gacagcgttg acgccccatc caccgcaaaa acaggcgat ggtgtgacat taccacttc
1141 agtgcgtgtt atcgccggac acgatactaa tctggcaaat ctggcgccg cactggagct
1201 caactggacg ctccccgtc agccggataa cacgccgcca ggtggtgaac ttgtgttga
1261 acgtggcgt cggctaagcg ataacagcca gtggattcag gtttcgctgg tctccagac
1321 ttacagcag atgcgtgata aaacgcgct gtcattaaat acgccgccg gagagggtga
1381 actgacctg gcaggatgtg aagagcgaaa tgcgcagggc atgtgttcgt tggcaggtt
1441 tacgcaaatc gtgaatgaag cagcatacc ggcgtgcagt ttgtaatgca taaaaagag
1501 cattcagtta cctgaatgct ctgaggctga tgacaaacga agaactgtct aatgcgtaga
1561 ccggaanaagg cgttacgcc gcatccggcc acttcagtt ttcctcttc tggagtaac
1621 tataaccgta atagtatat ccgtaactgt aagcgggtgt ggcgcgttta atcacacat
1681 tgaggatagc gcccttaata ttgacgcctg cctgttccag acgctgcatt gacaaactca
1741 cctctttggc ggtgttcaag ccaaaacgcg caaccagcag gctggtgcca acagaacgcc
1801 ccacgacgc ggcacactc accgccagca tggcgccgt atcgacaac accagatcgt
1861 aatggtcgtt cgcctatcc agtaattgac gcatccgatc g

Figure 7b

1 taaggagcag aaacaatgtg gtatttactt tgggtcgtcg gcattttgtt gatgtgttcg
 61 ctctccacc ttgtgttgg atggttgac ccgcgtctga aaagttaacg aacgtaggcc
 121 tgatgcggcg cattagcacc gcatcaggca atcaataatg tcagatatga aaagcggaaa
 181 catatcgatg aaagcgatct taatccatt tttatctctt ctgattccgt taaccccgca
 241 atctgcattc gctcagagtg agccggagct gaagctggaa agtgtgtgga ttgtcagtcg
 301 tcattggttg cgtgtccaa ccaaggccac gcaactgatg caggatgca cccagacgc
 361 atggccaacc tggccggtaa aactgggttg gctgacaccg cnggtgtg agctaategc
 421 ctatctcgga cattaccaac gccagcgtct ggtagccgac ggattgctgg cgaaaaagg
 481 ctgcccgcag tctgggtcagg tccgattat tctgtatgac gacgagcgta cccgtaaaa
 541 aggcgaagcc ttcgcccgcg ggtggcacc tgactgtgca ataaccgtac ataccaggc
 601 agatacgtcc agtcccgatc cgttatitaa tctctaaaa actggcgtt gccactgga
 661 taacgcgaac gtgactgacg cgtatctcag cagggcagga gggtaattg ctgacttac
 721 cgggcacggg caaacggcgt ttcgcgaact ggaacgggtg cttaatttc cgaatcaaa
 781 cttgtgcctt aaacgtgaga aacaggacga aagctgttca ttaacgcagg cattaccat
 841 ggaactcaag gtgagcggcg acaatgtctc attaacgggt gcggttaagcc tgcacat
 901 gctgacggag atattctcc tgcaacaagc acagggaatg ccggagccgg ggtggggaag
 961 gateaccgat tcacaccagt ggaacacett gctaagttg cataacgcgc aattttatt
 1021 gctacaacgc acgccagagg ttcccgcag ccgcgccacc ccgttattag atttgatcaa
 1081 gacagcgttg acgcccac caccgcaaaa acaggcgtat ggtgtgacat taccacttc
 1141 agtgcgttt atcgcgggac acgatactaa tctggcaaat ctggcgggcg cactggagct
 1201 caactggacg ctcccgggtc agccggataa cagcccgcca ggtgtgtaac tgggtttga
 1261 acgtggcgt cggctaagcg ataacagcca gtggattcag gtttgcgtgg tctccagac
 1321 ttacagcag atgcgtgata aaacgcgct gtcattaaat acgcgcgccg gagagggtgaa
 1381 actgacctg gcaggatgtg aagagcgaaa tgcgcagggc atgtgttcgt tggcaggtt
 1441 tacgcaaatc gtgaatgaag cagcatacc ggcgtgcagt ttgtaatgca taaaaagag
 1501 cattcagtta cctgaatgct ctgaggctga tgacaaacga agaactgtct aatgcgtaga
 1561 ccggaaaagg cgttcagcc gcatccggcc acttcagtt ttcctcttc tggagtaac
 1621 tataaccgta atagttag ccgtaactgt aagcgggtgt ggcgcgttta atcacaccat
 1681 tgaggatagc gctttaata ttgacgctg cctgttccag acgtgcatt gacaaactca
 1741 cctctttggc ggtgttcaag ccaaacgcg caaccagcag gctgggtcca acagaacgc
 1801 ccaagaccgc ggcacactc accgcagca tggcggcgt atcgacaatc accagatcgt
 1861 aatggtcgtt cggccattcc agtaattgac gcatccgac g

Figure 8

Amino acid sequence for E. coli appA (wild type) (SEQ ID NO:8)

MKAILIPFLSLLIPLTPQSAFAQSEPELKLESVVIVSRHGVRAPTKATQLMQDVT
 PDAWPTWPVKLGWLTPRGGELIAYLGHYQRQRLVADGLLAKKGCPQSGQV
 AIIADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLFNPLKTGVCQLD
 NA
 NVTDAILSRAGGSIADFTGHRQTAFRELERVLNFPQSNLCLKREKQDESCSLT
 QALPSELKVSADNVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQW
 NTLLSLHNAQFYLLQRTPEVARSRATPLLDLIKALTTPHPPQKQAYGVTLPSTV
 LFIAGHDTNLANLGGALELNWTLPGQPDNTPPGGELVFERWRRLSDNSQWVQ
 VSLVFQTLQQMRDKTPLSLNTPPGEVKLTLAGCEERNAQGMCSLAGFTQIVN
 EARIPACSL

Bold-Underlined amino acid residues are shown below in the modified appA enzyme
 (SEQ ID NO:10)

MKAILIPFLSLLIPLTPQSAFAQSEPELKLESVVIVSRHGVRAPTKATQLMQDVT
 PDAWPTWPVKLGELTPRGGELIAYLGHYWRQRLVADGLLPKCGCPQSGQVA
 IIAADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLFNPLKTGVCQLDN
 A
 NVTDAILEAGGSIADFTGHYQTAFRELERVLNFPQSNLCLKREKQDESCSLTQ
 ALPSELKVSADCVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWN
 TLLSLHNAQFDLQRTPEVARSRATPLLDLIKALTTPHPPQKQAYGVTLPSTVLF
 IAGHDTNLANLGGALELNWTLPGQPDNTPPGGELVFERWRRLSDNSQWVQS
 LVFQTLQQMRDKTPLSLNTPPGEVKLTLAGCEERNAQGMCSLAGFTQIVNEA
 RIPACSL